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Full title: Genetic and environmental contributions to the association between mood disorder and periodontal disease: a cross-sectional study amongst female twins in the UK

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ABSTRACT

Aim

This study aimed to investigate the factors associated with periodontal traits considering genetic and environmental background in predominantly older female twins.

Methods

This was a cross-sectional study using self-reported questionnaires for periodontal traits in TwinsUK. Age-adjusted and age-stratified multivariate analyses were conducted for all twins.

Subsequently, co-twin control analysis within genetically-identical twins who were discordant for periodontal traits was performed by controlling for genetic confounders.

Results

Data of twins aged 20-91 were available in 4143 individuals for self-reported periodontitis and 4244 for gum bleeding. Age-adjusted model showed increasing risk in: smoking, anxiety/stress and depression for both periodontal traits. Within discordant monozygotic twins (514 individuals for periodontitis and 754 for gum bleeding), the association of anxiety/stress remained significant both for periodontitis (OR 1.60, CI 1.02-2.52) and gum bleeding (OR 1.60, CI 1.06-2.40). A significant relationship for depression remained for periodontitis (OR 1.68, CI 1.04-2.70), but it was no longer significant for gum bleeding. Age-stratification showed that the association of mood disorders with periodontal traits was generally stronger in older group.

Conclusions

Multivariate analysis among discordant monozygotic female twins found mood disorders were independently associated with periodontal traits, suggesting that genetic / early life environmental factors may not explain this association.

Clinical Relevance

Scientific rational for study

Understanding genetic and environmental confounding is fundamental to consider health problems.

Principal findings

Some health problems such as cardiovascular disease and diabetes have been established the relationship with periodontal disease, whereas mood disorders were not shed light on its clinical significance. This study alarmed to pay more attention to behavioural patterns for periodontal patients who have mood disorder.

Practical implications

This study including female twins highlights that not genetic and familial environment, but adulthood environment may play an important role to explain the association between mood disorder and periodontal disease.

INTRODUCTION

Chronic diseases including periodontal disease are a growing burden amongst older population as life expectancy increases. Periodontal disease results from a combination of genetic and environmental risk factors. Classical twin studies have demonstrated the extent of genetic and environmental

contributions to the susceptibility to periodontal diseases (Michalowicz et al., 2000). These have shown that the heritability of periodontal disease is generally intermediate (approximately 30%). Recently, a number of larger genome-wide association studies of periodontal disease have been reported but results from different cohorts show markedly different results (Schaefer et al., 2010), (Feng et al., 2014), (Munz et al., 2017).

A large number of epidemiological surveys have been conducted to determine particular environmental risk factors (Borrell et al., 2006, Zini et al., 2011). Age, gender, smoking and socio-economic status are all considered to be important risk factors. There is growing evidence that a number of systemic conditions such as obesity (Boesing et al., 2009), cardiac diseases (Beck and Offenbacher, 2005, Loos et al., 2000), hypertension (Martin-Cabezas et al., 2016), psychosocial and psychiatric factors (Vettore et al., 2003, Johannsen et al., 2006) are associated with periodontitis. However, as far as we know, there are still few studies that have fully clarified the association between these conditions and periodontal traits together, in the same dataset.

Furthermore, it is unclear how these modifiable risk factors link to periodontitis when considering genetic factors. So called ‘environmental factors’ themselves have a genetic component (Pallister et al., 2015, Boardman et al., 2010). It is important to determine whether individual environmental risk

factors for periodontitis are independent of genetic factors or not. Identical twins who are discordant for periodontal conditions enable the examination of the possible impact of environmental factors independent of genetics.

In this study, we examined the association of periodontal traits with oral-health risk factors as independent covariates. We then examined whether these associations were still present when accounting for the shared genetic and environmental backgrounds of twins.

MATERIALS AND METHODS

Study population

Participants were enlisted in the TwinsUK registry, which consists of volunteer twins who were not selected for inclusion by any other criteria; however, females have exceeded males, and monozygotic (MZ) number exceeded dizygotic (DZ), in part because the registry was started to study principally diseases which were more prevalent in, or unique to, females. The participants in our study predominantly consist of older-age twins from 20 to 91 years old (Table 2). The TwinsUK registry is one of the largest adult twin cohorts encompassing multivariate medical phenotypes in the world. All the participants provided informed consent before the examinations. We made two datasets, for the two phenotypes, self-reported periodontitis and gum bleeding. Zygosity determination was used by

standard questionnaire (Martin and Martin, 1975) and confirmed by DNA short-tandem repeat fingerprinting.

Periodontal phenotypes

Dental questionnaires have been conducted within this cohort since 2004 with some question modification in 2015. The information on gum bleeding and tooth mobility was collected by written questionnaire, and to maximize the power of the study, all available information was used. The questions “Have you ever had the condition of gum bleeding” (2004-2014) and “Do your gums bleed when you brush your teeth?” (2015-2016) were merged to produce a single phenotype. The most recent data were used in case of multiple responses. For periodontitis, “Have you ever had the condition of gum decay or loose teeth” (2004-2014) and either “Do you have any loose teeth” (2015-2016) or “Have you ever been told by a dentist or hygienist that you have gum disease?” (2015-2016) were merged in a similar manner. This produced two binary traits, bleeding gums and periodontitis, which were used for subsequent analyses.

As part of a larger validation study of self-reported periodontal disease in this cohort, 80 individuals received a full dental examination and there periodontal status was classified as healthy, gingivitis, mild, moderate and severe periodontitis according to the case definitions described by Page

and Eke (Page and Eke, 2007). Two binary classifications (moderate /severe periodontitis and gingivitis) and the original examination records (categorical periodntal status wih four grades and the percentage of bleeding on probing (BOP)) were tested against the self-reported periodontitis trait and gum bleeding respectively.

Health conditions

Since 2004, general health questionnaires have been undertaken. To obtain information on anxiety/stress disorder, depression, coronary heart disease, and hypertension including high blood pressure, we created binary summary variables (Table1). Obesity was defined by a BMI which was 30 or higher. We also measured carotid intima-media thickness using ultrasonography in four different parts which of anterior left, anterior right, posterior left, and posterior right sides. We set the cut-off as 1.0mm (Chambless et al., 1997), and classified two groups. Subjects with at least one site measuring over 1.0mm was grouped in arteriosclerosis positive, and the others were grouped arteriosclerosis negative.

Dietary questions

Self-reported Food Frequency Questionnaires were completed by all the participants following European Prospective Investigation of Cancer Norfolk guidelines . From this dataset, we created the Healthy Eating Index (HEI) according to guidelines set out by Guenther and co-workers 2010 (Guenther et al., 2013). The HEI is comprised of 12 weighted components as listed on the USDA website (Developing the Healthy Eating Index). The components are summed together to create the total HEI score. The maximum score is 100, and a higher score represents a healthier diet. HEI data in our study showed a normal distribution covering a wide range of diets (scores ranged between 28 and 93).

Smoking, education and income

All the participants were classified into three categories of smoking behaviour ("Never", "Former", and "Current" smoker). Household income (three groups; <£25,000, £25,000-£75,000 and >£75,000) and final year of formal education (three groups; <17, 17-20, and >20 years) were used to characterize socio-economic status.

Statistical Analysis

Firstly, we tested the hypothesis that each periodontal risk factor would be associated with either status of self-reported periodontitis or gum bleeding among all participants. Only factors significant in the univariate analysis were included in multivariate analyses using Generalized Linear Mixed-Effects Models with either self-reported periodontitis or gum bleeding as the dependent variable. Anxiety/stress disorder and depression were separately introduced into a multivariate model because of a multicollinearity (p-value < 0.001 produced by chi-square test). Generalized Linear Mixed-Effects Models is conceptually equivalent to a matched case control design, as it can control the clustering of twins within a pair. This design compared all identified cases of dental diseases with all available controls who had a healthy oral condition, and treating participants as individuals rather than as members of twin pairs. Age stratified analysis using mean age as the cut-off was applied in order to examine whether the significant associations found in the age adjusted model were consistent in both young and old groups.

Secondly, we used a co-twin control design by identifying a sample of MZ twins discordant for the dental disorders and comparing affected and unaffected twins. This design controls for genetic factors, under the assumption that MZ twins share 100% of their segregating genes. A significant association

in this model indicates a direct association that cannot be explained by genetic factors (Carlin et al., 2005). In all the analyses, $P < 0.05$ was considered to denote a statistically significant difference.

RESULTS

The total participants in the dataset for self-reported periodontitis and gum bleeding were 4683 (89.4% female) and 4805 (89.3% female) respectively. Due to small numbers of male twins in this registry, analyses were therefore restricted to adult females only. Therefore, the final set of self-reported periodontitis data contained 2330 MZ and 1813 DZ individuals, and that of gum bleeding contained 2376 MZ and 1868 DZ individuals. Validation study resulted that unadjusted responses to self-reported periodontitis had a specificity of 1 (positive predictive value of 1), and sensitivity of 0.28 against the binary periodontal classification. Area under the ROC curve (AUC) against the categorical periodontal classification was 0.78. With regard to the validation of gingivitis, the self-reported gum bleeding had a specificity of 0.75 and sensitivity of 0.48 against the binary gingivitis classification. Area under the ROC curve (AUC) against the continuous gingivitis value using the percentage of BOP was 0.78.The distribution of MZ and DZ twins is shown in Table 2. In total, 20.8% of the cohort had periodontitis and 45.7% had gum bleeding. Interclass correlation for self-reported periodontitis was (MZ 0.21, DZ 0.06) and that of gum bleeding was (MZ 0.29, DZ 0.17).

Falconer's formula (Falconer D. S., 1996) therefore generated the heritability of 30% for self-reported periodontitis and 24% for gum bleeding. F-test (for age and HEI) and chi-squared test (for the rest of the factors listed in Table2) showed that there are statistically significant differences between some factors (age, smoking, income, education and hypertension) and zygosity for both periodontal traits.

Table 3 shows the results of the univariate association between either self-reported periodontitis or gum bleeding and other health conditions. In age adjusted multivariate model (Table 4), self-reported periodontitis was significantly associated with smoking (OR former smokers 1.61 (1.36-1.90) current smokers 1.67 (CI 1.32-2.12)) , obesity (OR 1.28 (CI 1.05-1.56)), anxiety/stress (OR 1.39 (CI 1.13-1.70)), and Healthy Eating Index (OR 0.99 (CI 0.98-0.99)) while gum bleeding was significantly associated with former smoker compared to non-smoker (OR 1.19 (CI 1.04-1.36)), lower income compared to middle income (OR 0.85, (CI 0.74-0.97)), and anxiety/stress disorder (OR 1.47 (CI 1.24-1.74)). Anxiety/stress disorder was one of the consistent factors which significantly related to two periodontal traits. Age stratified analysis, revealed that for periodontitis, this association was still significant in the older group (OR 1.51 (CI 1.09-2.08), p-value = 0.01) whereas it was not significant in the younger age group (OR 1.28 (CI 0.90-1.82), p-value = 0.16); on the other hand, the significance of anxiety/stress disorder held in both younger and older groups for gum bleeding. A similar trend was

found in models replacing anxiety/stress disorder with depression (Table5). The significant association between depression and both periodontal traits held in both younger and older groups.

There were 258 pairs (516 individuals) of MZ twins discordant for self-reported periodontitis. Testing for associations of the above risk factors between these pairs, the presence of anxiety/stress disorder and healthy eating index were significantly associated with self-reported periodontitis with the odds ratio 1.60 (p-value = 0.02) and 0.98 (p-value = 0.047) respectively (Table 4). In the multivariable model introducing depression instead of anxiety/stress disorder to independent variables, depression was only significantly associated with self-reported periodontitis trait (OR = 1.68; p-value = 0.01; Table 5). Other variables were not significantly associated when applying multivariate regression models for periodontitis-discordant MZ twins to control for factors shared by identical twins. The significant association between anxiety/stress disorder and periodontitis remained even amongst the periodontitis-discordant MZ twins in older group (OR = 1.51; p-value = 0.02).

For gum bleeding, 377 MZ pairs (754 individuals) were found to be discordant. Co-twin control analysis among MZ twins who were discordant for gum bleeding trait showed that only the presence of anxiety/stress disorder was significantly associated with gum bleeding with the odds ratio 1.60 (p-value 0.02) in Table 4. The association between depression and gum bleeding was not significant among gum bleeding discordant MZ twins (Table 5).

DISCUSSION

The striking result was that, in multivariate analysis, both mood disorders, anxiety/stress and depression were strongly related with both self-reported periodontal traits even controlling for risk factors and previously reported systemic health problems. Although, no statistical significance was found in the relationship between anxiety/stress disorder and periodontitis in the younger age group, the relationship between mood disorder and self-reported periodontal traits was nominally stronger in older versus younger age. This might indicate that older people are more vulnerable to both mood disorder and periodontal disease. It may also imply that genetic sesceptibility is one of the main reasons for the strong link between anxiety/stress disorder and periodontal disease in younger age, however, this is because of the contribution to the disease development in older age. The association of cardiovascular disease with periodontitis has, to date, received more attention than that of mood disorders. On the other hand, there is recent rising public health and research interest in mental health including mood disorder as a driver of systemic disease. This study underlines the need to consider psychological factors in the field of periodontology and geriatric dentistry. At the same time, periodontal disease is likely to be overlooked particularly among people with psychological illness. Therefore, it is important to investigate the temporal association and the mechanism behind the association where current knowledge is limited. Two broad hypotheses are present in the literature –

altered immune reaction driven by anxiety and depression, or behavioral change leading to change in oral hygiene practices and smoking. The first hypothesis is that psychiatric disorders trigger an over active inflammatory response to stressors and pathogens (Warren et al., 2014), for example in the periodontal tissues. Poor oral health has been reported as a risk factor for depression (Yamamoto et al., 2017) (Hsu et al., 2015). Some researchers have already reported certain mediators which have strong associations between these diseases (Mousavijazi et al., 2013). A number of studies have demonstrated the negative impact of periodontal disease on quality of life, which might contribute to the development of psychological illness (Durham et al., 2013, Needleman et al., 2004, Buset et al., 2016).

Alternatively, one of the reasons why mental health disorders are crucial for oral health, may be because sufferers change their behavior and their environment dramatically. Goyal at et al. reported several behavioral changes ensuing from psychological disorders (Goyal et al., 2013). For instance, coping type such as avoidance strategy and social support was also strongly associated with mood disorder (Del Rosso et al., 2013). Anxiety/stress or depression may lead to periodontal disease through neglect of oral hygiene, changes in diet, increasing smoking and other risk behaviors, induction of oral habits, alteration of salivary condition, or endocrine imbalances (Croucher et al., 1997). While our results do not test the direction of association, our study implies that environmental

factors are likely to explain the association. Co-twin control analyses suggested that factors shared by MZ twins (genetic / early life environment) do not explain the association between anxiety/stress and periodontal disease among female adults. In fact, in our analysis, both mood disorders, anxiety/stress and depression were highlighted as the only risk factors which remained significant within MZ twin pairs who share genetic and some environmental factors which could confound other association studies.

Our analysis also investigated the role of other factors previously reported as associated with periodontal disease. Throughout univariate analysis among the population of both MZ and DZ twins, demographical information including age and socio-economic status showed opposing trends for the two traits, self-reported periodontitis and gum bleeding. Young age and high socio-economic status were associated with gum bleeding, whereas old age and low socio-economic status were associated with self-reported periodontitis. This feature is consistent with the natural history of periodontal disease progression in which self-reported periodontitis, including the feature of tooth mobility (more severe stage of periodontal disease) is generally preceded by gum bleeding; therefore, gum bleeding is detectable in a younger population, whereas self-reported periodontitis becomes more apparent with age. The univariate results suggested that the both periodontal traits represent different nature of these traits. Self-reported periodontitis may reflect severe periodontitis while gum bleeding may suggest

either gingivitis, early stage periodontitis, and therefore be a less clinically relevant phenotype. Multivariate regression analysis also identified a negative association between the healthy dietary habits and severe periodontitis when controlling for genetic factors. The potential link here is that the significant deterioration of oral health leading to loose teeth results in restriction of food choice and therefore imbalanced diet. We also found that former smoker and middle income adults in young population were significant risk factors for bleeding gums. The possible reason why current smokers were not significantly associated with bleeding while former smokers were, is that whilst current and previous smoking have been widely associated with greater severity and extent of periodontitis, it is widely believed that there is a reduction in marginal bleeding amongst current smokers, an effect which disappears after smoking cessation by suppressing the inflammatory response but which may modify clinical signs of disease before that (Dietrich et al. J Periodontol. 2004; Al-Bayaty et al. Biomed Res Int. 2013). One of the plausible reasons why smoking status did not show the association with periotontitis trait within discordant monozygotic twins could be that these factors are insufficiently variant within identical twins to have the detective power with this sample size. According to the power calculation to achieve 80% power, the estimated number of samples was 2579 for gum bleeding trait and 346 for periodontitis trait. Secondly, there might be the factors which shared by identical twins (genetic and familial environmental factors) attenuate the

association between smoking and periodontal disease. In other words, there might be mutual genetic and familial environmental factors between smoking and periodontitis trait.

There are a number of limitations need to be considered. One of the main shortcomings of this study is that all the phenotypes were obtained by self-reported questionnaires rather than clinical examination. Self-report is likely to underestimate presence of mental health disorders, which would most likely serve to diminish any association found. Considering the binary traits of anxiety/stress disorder and depression, we conducted the cross-referencing test by using a subset of the cohorts in order to test whether anxiety/stress or mood symptoms showed the same effects. In this analysis, we used 415 individuals who also had completed the Hospital Anxiety and Depression Scale (HADS) in 2000. This scale among the anxiety/stress disordered group (mean 7.52) was significantly higher than that of the unaffected group (mean 5.68) (t test, p-value<0.001). Likewise, the HAD score of depression positive group (mean 5.51) was significantly higher than that of negative group (mean 3.01) (t test, p-value<0.001). Subsequently, the validation of both anxiety and depression traits were tested against the self-reported measures. The criteria of HADS standardized anxiety and depression was defined by the HAD scale over 7. The result showed relatively high specificity (anxiety 0.88, depression 0.91) despite low sensitivity (anxiety 0.15, depression 0.63).

As regards the use of self-reported periodontal questionnaires, equally, the validation study showed low sensitivity, which suggest that some people might either not notice they have gum bleeding and tooth mobility, or forget the history of periodontitis which diagnosed by dentists/hygienist. In addition, teeth which were previously mobile due to periodontitis may have been extracted before the study assessment. According to the validation test using the categorical periodontal classification, we suggest that this classification tends to have a wide range of periodontal status in the moderate group so that some of the subjects who answered negative for our questionnaire could have periodontal condition which is very close to mild, but classified moderate. However, our study as well as a number of previous studies have demonstrated that combinations of the 2 questions used in this study, namely loose teeth and professional diagnosis of gum disease can achieve high positive predictive value for moderate to severe periodontitis (Page and Eke, 2007, Eke et al., 2013), although rather lower sensitivity rates suggesting that whilst the self-reported periodontitis measure used here is valid, it is likely to markedly underestimate the total number of actual cases of periodontitis in this cohort (Eke et al., 2013, Heaton et al., 2017, Chatzopoulos et al., 2016, Dietrich et al., 2009) and compound reporting biases. However, there is little information of the accuracy of self-reported bleeding, and thus the phenotype of "gum bleeding" may be less robust than the presence of self-reported periodontitis.

We designed the classical twin model to eliminate genetic and familial environmental factors by using genetically identical twins. This twin model assumes that MZ twins are genetically identical, but post-zygotic mutation events can occur. Somatic point mutations during early development occur with a frequency of 1.2×10^{-7} per base pair per twin pair (Li et al., 2014). However, it is still unknow whether these tiny differences have phenotypic consequences and to what extent. The classical twin model, we used in the present study was not able to consider not only these mosaicisms but also gene regulatory mechanism including epigenetic modifications. These should not be ruled out as these genetic regulatory mechanisms may contribute to pathophysiology in human health traits including mood disorder and periodontal disease. Although, this study found that genetic and early-life environmental factor might not explain the association between mood disorder and periodontal disease, we still need to keep in mind that gene regulatory mechanism might play a role in development of this association.

Longitudinal studies are needed to confirm a clear picture of the mechanism between mood disorder and periodontal disease particularly among older population. Furthermore, conclusions may not be applicable to all populations because only females living in UK were included in this study and aetiology might be different between gender. Nevertheless, our present study suggests that the

associations between mental health disorders and periodontal condition are strong even when controlling for potential risk factors, systemic diseases and genetic confounding.

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Tables

Table 1. Questionnaire for general health

Traits	Questionnaire
Anxiety / Stress	Have you ever been told by doctor or other health professional that you had: Anxiety or stress disorders?
Depression	Have you ever told by a doctor or other health professional that you had depression?
Coronary Heart disease	Have you ever been told by doctor or other health professional that you had coronary heart disease?
Hypertension (High blood pressure)	Have you ever been told by doctor or other health professional that you had hypertension (high blood pressure)?

Table 2. The general distribution of each monozygotic (MZ) and dizygotic (DZ) twins

		Periodontitis		Gum bleeding	
		MZ (N=2330)	DZ (N=1813)	MZ (N=2376)	DZ (N=1868)
Continuous variables [a)]					
Mean age		55.7	59.4	55.5	59.2
Mean HEI[#]		60.4	60.6	60.9	60.9
Categorical variables [b)]					
Periodontitis	0 (Negative)	1858 (80%)	1424 (79%)	-	-
	1 (positive)	472 (20%)	389 (21%)	-	-
Gum bleeding	0 (Negative)	-	-	1263 (53%)	1040 (56%)
	1 (Positive)	-	-	1113 (47%)	828 (44%)
Smoking	0 (Never)	1363 (59%)	956 (53%)	1385 (58%)	990 (53%)
	1 (Former)	706 (30%)	619 (34%)	711 (30%)	634 (34%)
	2 (Current)	259 (11%)	230 (13%)	278 (12%)	237 (13%)
Income	0 (Lowest)	1043 (45%)	921 (50%)	1044 (44%)	939 (50%)
	1 (Middle)	1035 (44%)	755 (42%)	1085 (46%)	777 (42%)
	2 (highest)	252 (11%)	137 (8%)	247 (10%)	152 (8%)
Education	0 (Lowest)	997 (43%)	934 (51%)	993 (41%)	958 (52%)
	1 (Middle)	745 (32%)	538 (30%)	776 (33%)	549 (29%)
	2 (Highest)	588 (25%)	341 (19%)	607 (26%)	361 (19%)
Obesity	0 (BMI<30)	1936 (84%)	1403 (78%)	1969 (83%)	1450 (78%)
	1 (BMI>=30)	380 (16%)	393 (22%)	393 (17%)	401 (22%)
Anxiety / Stress	0 (Negative)	1974 (85%)	1576 (87%)	1998 (84%)	1602 (86%)
	1 (Positive)	356 (15%)	237 (13%)	378 (16%)	266 (14%)
Depression	0 (Negative)	1978 (86%)	1558 (87%)	2010 (86%)	1597 (87%)
	1 (Positive)	319 (14%)	234 (13%)	333 (14%)	246 (13%)
CHD[#]	0 (Negative)	2225 (95%)	1717 (95%)	1618 (96%)	1303 (95%)
	1 (Positive)	105 (5%)	96 (5%)	76 (4%)	64 (5%)
Arteriosclerosis	0 (Negative)	2218 (95%)	1731 (95%)	2253 (95%)	1780 (95%)
	1 (Positive)	112 (5%)	82 (5%)	123 (5%)	88 (5%)
Hypertension	0 (Negative)	1868 (80%)	1345 (74%)	1910 (80%)	1389 (74%)
	1 (Positive)	462 (20%)	468 (26%)	466 (20%)	479 (26%)

a) The number in the table shows the mean value of each category.
b) The number in the table shows the number of individuals in each category
CHD; Coronary heart disease, HEI; Healthy eating index

Table 3. Univariate association between basic and health factors and periodontal traits

		Periodontitis			Gum bleeding		
		Negative (N=3282)	Positive (N=861)	P-value [c]	Negative (N=2303)	Positive (N=1941)	P-value [c]
Continuous variables [a]							
Age	Mean	56.7	59.6	4.7e-11*	58.4	55.6	1.5e-12*
HEI [#]	Mean	60.8	59.4	9.4e-4*	60.8	61.0	0.73
Categorical variables [b]							
Smoking status	Never Former Current	1923 988 (30%) 363 (11%)	396 337 (39%) 126 (15%)	2.6e-10*	1318 692 (30%) 288 (13%)	1057 653 (34%) 227 (12%)	0.04*
Income	Low Middle High	1533 1435 (44%) 314 (19%)	431 355 (40%) 75 (9%)	0.21	1150 950 (41%) 203 (9%)	833 912 (47%) 196 (10%)	2.9e-5*
Education	Low Middle High	1507 1017 (31%) 758 (23%)	424 266 (31%) 171 (20%)	0.09	1105 701 (30%) 497 (22%)	846 624 (32%) 471 (24%)	0.01*
Obesity	Negative Positive	2681 577 (18%)	658 196 (23%)	5.8e-4*	1849 436 (19%)	1570 358 (19%)	0.70
Anxiety / Stress	Negative Positive	2843 439 (13%)	707 154 (18%)	9.4e-4*	2007 296 (13%)	1593 348 (18%)	5.4e-6*
Depression	Negative Positive	2845 405 (12%)	691 148 (17%)	1.2e-4*	2006 268 (12%)	1601 311 (16%)	3.5e-5*
CHD [#]	Negative Positive	3130 152 (5%)	812 49 (6%)	0.23	2207 96 (4%)	1841 100 (5%)	0.15
Arteriosclerosis	Negative Positive	3140 142 (4%)	809 52 (6%)	0.04*	2201 102 (4%)	1832 109 (6%)	0.09
Hypertension	Negative Positive	2584 698 (21%)	629 232 (27%)	4.5e-4*	1783 520 (22%)	1516 425 (22%)	0.62

a) The number in the table shows the mean value of each category.
b) The number in the table shows the number of individuals in each category
c) P-values were generated by t-test for continuous variables and chi-square test for categorical variables in the univariate association between basic and health factors and periodontal traits.
HEI; Healthy eating index, CHD; Coronary heart disease

Table 4. The multivariate association for periodontal traits with having anxiety/stress disorder in the independent variable

		Odds ratio for periodontitis					
		Adjusted by age		Age stratified			
				Younger (age <58)		Older (age >57)	
		All participants (N*= 4143)	Discordant MZ twins (N= 516)	All participants (N= 1898)		All participants (N= 2245)	Discordant MZ twins (N= 268)
Age		**1.02 (1.01, 1.02) p = 2.4e-8**	1.00 (0.99, 1.02) p = n.s.	-		-	-
Smoking	Former	**1.61 (1.36, 1.90) p = 1.3e-7**	1.33 (0.89, 1.99) p = n.s.	**1.79 (1.34, 2.40) p = 9.8e-5**		**1.61 (1.26, 2.07) p = 1.8e-4**	1.45 (0.83, 2.54) p = n.s.
	Current	**1.67 (1.32, 2.12) p = 6.9e-5**	1.11 (0.65, 1.92) p = n.s.	**1.55 (1.06, 2.27) p = 0.02**		**1.85 (1.26, 2.73) p = 1.8e-3**	1.15 (0.52, 2.55) p = n.s.
Income	Low	-	-	-		-	-
	High	-	-	-		-	-
Education	Middle	-	-	-		-	-
	High	-	-	-		-	-
Obesity		**1.28 (1.05, 1.56) p = 0.02**	0.97 (0.59, 1.60) p = n.s.	**1.45 (1.03, 2.05) p = 0.03**		1.22 (0.91, 1.64) p = n.s.	0.86 (0.44, 1.69) p = n.s.
Anxiety / Stress		**1.39 (1.13, 1.70) p = 3.1e-3**	**1.60 (1.02, 2.52) p = 0.02**	1.28 (0.90, 1.82) p = n.s.		**1.51 (1.09, 2.08) p = 0.01**	**1.51 (1.12, 3.95) p = 0.02**
Arteriosclerosis		1.05 (0.73, 1.51) p = n.s.	4.23 (0.86, 20.8) p = n.s.	0.69 (0.33, 1.45) p = n.s.		1.31 (0.80, 2.17) p = n.s.	1.49 (0.56, 3.93) p = n.s.
Hypertension		1.14 (0.95, 1.38) p = n.s.	0.99 (0.63, 1.54) p = n.s.	**1.66 (1.14, 2.42) p = 8.4e-3**		1.04 (0.81, 1.35) p = n.s.	0.97 (0.51, 1.49) p = n.s.
HEI*		**0.99 (0.98, 0.99) p = 9.9e-4**	**0.98 (0.97, 0.99) p = 0.05**	0.99 (0.98, 1.00) p = n.s.		**0.98 (0.97, 0.99) p = 4.0e-3**	0.98 (0.96, 1.01) p = n.s.

Odds ratio for gum bleeding			
Adjusted by age		Age stratified	
All participants (N= 4244)	Discordant MZ twins (N= 754)	Younger (age <58) All participants (N= 1978)	Older (age >57) All participants (N= 2266)
0.99 (0.98, 0.99) p = 1.23e-7	0.99 (0.99, 1.01) p = n.s.	-	-
1.19 (1.04, 1.36) p = 0.02	1.01 (0.74, 1.40) p = n.s.	1.09 (0.86, 1.37) p = n.s.	**1.31 (1.09, 1.57) p = 6.3e-3**
0.91 (0.75, 1.11) p = n.s.	0.81 (0.51, 1.30) p = n.s.	0.76 (0.56, 1.02) p = n.s.	1.10 (0.82, 1.47) p = n.s.
0.84 (0.72, 0.99) p = 0.03	1.00 (0.72, 1.38) p = n.s.	0.98 (0.78, 1.23) p = n.s.	0.84 (0.70, 1.02) p = n.s.
0.96 (0.74, 1.24) p = n.s.	0.88 (0.54, 1.41) p = n.s.	0.95 (0.70, 1.28) p = n.s.	1.04 (0.69, 1.57) p = n.s.
1.05 (0.91, 1.21) p = n.s.	0.93 (0.66, 1.31) p = n.s.	**0.74 (0.58, 0.95) p = 0.02**	**1.29 (1.05, 1.57) p = 0.02**
1.03 (0.87, 1.22) p = n.s.	1.06 (0.71, 1.60) p = n.s.	0.81 (0.62, 1.05) p = n.s.	1.28 (0.99, 1.64) p = n.s.
-	-	-	-
1.47 (1.24, 1.74) p = 2.7e-5	**1.60 (1.06, 2.40) p = 0.02**	**1.68 (1.26, 2.22) p = 3.5e-4**	**1.36 (1.07, 1.72) p = 0.02**
-	-	-	-
-	-	-	-
-	-	-	-

* HEI; Healthy eating index, N; The number of individuals

Table 5. The multivariate association for periodontal traits with having depression disorder in the independent variable

		Odds ratio for periodontitis			
		Adjusted by age		Age stratified	
				Younger (age <58)	Older (age >57)
		All participants (N*= 4143)	Discordant MZ twins (N= 516)	All participants (N= 1898)	All participants (N= 2245)
Age		**1.02 (1.01, 1.03) p =2.8e-8**	1.00 (0.99, 1.02) p = n.s.	-	-
Smoking	Former	**1.64 (1.36, 1.98) p = 3.5e07**	1.35 (0.91, 2.01) p = n.s.	**1.74 (1.29, 2.34) p = 2.7e-4**	**1.61 (1.265 2.08) p = 2.3e-4**
	Current	**1.56 (1.18, 2.05) p = 1.6e-3**	1.16 (0.67, 2.01) p = n.s.	**1.48 (1.01, 2.18) p = 0.04**	**1.79 (1.21, 2.66) p = 3.9e-3**
Income	Low	-	-	-	-
	High	-	-	-	-
Education	Middle	-	-	-	-
	Highest	-	-	-	-
Obesity		**1.30 (1.04, 1.63) p =0.02**	0.89 (0.54, 1.48) p = n.s.	1.42 (0.99, 2.01) p = n.s.	1.23 (0.91, 1.67) p = n.s.
Depression		**1.50 (1.18, 1.91) p = 8.8e-4**	**1.68 (1.04, 2.70) p = 0.03**	**1.49 (1.07, 2.08) p = 0.02**	**1.67 (1.18, 2.37) p = 4.2e-3**
Arteriosclerosis		0.95 (0.63, 1.44) p = n.s.	1.52 (0.62, 3.71) p = n.s.	0.69 (0.33, 1.44) p = n.s.	1.25 (0.74, 2.10) p = n.s.
Hypertension		1.08 (0.87, 1.33) p = n.s.	1.02 (0.65, 1.58) p = n.s.	**1.68 (1.14, 2.43) p = 0.01**	1.02 (0.78, 1.32) p = n.s.
HEI*		**0.99 (0.98, 0.99) p = 9.7e-4**	0.98 (0.97, 1.00) p = n.s.	0.99 (0.98, 1.00) p = n.s.	**0.98 (0.97, 0.99) p = 0.01**

Odds ratio for gum bleeding			
Adjusted by age		Age stratified	
		Younger (age <58)	Older (age >57)
All participants (N= 4244)	Discordant MZ twins (N= 754)	All participants (N= 1978)	All participants (N= 2266)
0.99 (0.98, 0.99) p = 3.5e-7	0.99 (0.99, 1.01) p = n.s.	-	-
1.21 (1.03, 1.41) p = 0.02	1.01 (0.73, 1.39) p = n.s.	1.07 (0.85, 1.35) p = n.s.	**1.36 (1.10, 1.70) p = 5.4e-3**
0.86 (0.68, 1.08) p = n.s.	0.81 (0.50, 1.31) p = n.s.	**0.72 (0.53, 0.97) p = 0.72**	1.06 (0.75, 1.51) p = n.s.
0.84 (0.72, 0.99) p = 0.04	0.98 (0.71, 1.36) p = n.s.	0.98 (0.80, 1.21) p = n.s.	1.84 (0.70, 1.02) p = n.s.
0.95 (0.73, 1.22) p = n.s.	0.83 (0.52, 1.34) p = n.s.	0.95 (0.73, 1.24) p = n.s.	1.04 (0.69, 1.57) p = n.s.
1.03 (0.86, 1.22) p = n.s.	0.93 (0.67, 1.31) p = n.s.	**0.74 (0.58, 0.95) p = 0.02**	**1.29 (1.01, 1.64) p = 0.04**
1.05 (0.86, 1.29) p = n.s.	1.05 (0.69, 1.58) p = n.s.	0.82 (0.63, 1.07) p = n.s.	1.33 (0.98, 1.79) p = n.s.
-	-	-	-
1.43 (1.17, 1.76) p =5.7e-4	1.31 (0.84, 2.04) p = n.s.	**1.34 (1.02, 1.76) p = 0.03**	**1.46 (1.07, 2.00) p = 0.02**
-	-	-	-
-	-	-	-
-	-	-	-

* HEI; Healthy eating index, N; The number of individuals